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Na^+/H^+ exchange inhibition improves post-transplant myocardial compliance in 4-hour stored donor hearts

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Na^+/H^+ exchange inhibitors have cardioprotective properties. The effects of the new Na^+/H^+ exchange inhibitor, HOE642 on myocardial function were assessed after transplantation of canine brain-dead and non-brain-dead donor hearts preserved for 4 h. Four groups were studied: brain-dead donors; non-brain-dead donors; brain-dead donors and recipients treated with HOE642 (2 mg/kg); and treated non brain-dead donors and recipients. Donor hearts were stored in NIH2. At the end of 60 min reperfusion after transplantation, pressure–volume curves were constructed. Biopsies were analysed histologically and ultrastructurally. Afterwards, weaning from cardiopulmonary bypass was accomplished. HOE642 improved compliance in hearts from both brain-dead and non-brain-dead donors. No differences in myocardial water content nor in myocardial performance were detected. No irreversible damage was seen ultrastructurally. It is concluded that myocardial compliance after transplantation was improved by administration of HOE642. The use of this inhibitor might improve the current myocardial preservation technique for transplantation. © 1998 The International Society for Cardiovascular Surgery

Keywords: experimental heart transplantation, heart preservation, myocardial compliance, Na^+/H^+ exchange inhibitors, brain death

Early allograft failure accounts for ± 20% of mortality in the first year after heart transplantation [1]. This early allograft failure might be reduced by better myocardial protection of the donor heart and better management of transplanted patients.

The successful return of myocardial function following cardiac transplantation depends largely on the quality of the donor organ and its preservation during transportation [2]. Optimal preservation not only allows for the recovery of myocardial function during reperfusion after transplantation, but also allows the heart to be transported over long distances, hence increasing the donor pool.

Myocardial ischaemia and reperfusion disturb cellular ionic homeostasis. The Na^+/H^+ exchange system is activated by tissue acidosis and induces intracellular Na^+ overload. This in turn activates the Na^+/Ca^{2+} exchange system causing intracellular Ca^{2+} overload [3–7]. Furthermore, increased intracellular Na^+ causes water absorption with potential myocardial contractile dysfunction [8]. The Ca^{2+} overload causes myocardial contracture, postischaemic dysfunction, arrhythmias and cell necrosis [5, 6, 9–12]. Myocardial oedema, and/or contractures have been commonly observed in ischaemic hearts, and have been shown to decrease ventricular compliance [8, 13–17], which may lead to the 'no reflow' phenomenon [18, 19] and consequently to poor graft function [8].

Inhibition of the Na^+/H^+ exchange system during ischaemia and reperfusion diminishes both Na^+ and Ca^{2+} overload and potentially prevents or delays myocardial damage [20–27]. In a previous study from our centre [25], it was shown that the Na^+/H^+

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inhibitor HOE694 improved postischaemic myocardial function in blood-perfused rabbit hearts. In that model it was shown that significantly less contracture developed during 45 min of global normothermic ischaemia and that during reperfusion myocardial-developed pressure and compliance were superior compared with that of untreated hearts when the Na^+/H^+ inhibitor was administered 20 min before the start of ischaemia. HOE642 (4-isopropyl-3-methylsulfonyl-benzoylguanidin-methanesuifonae) a new, selective and potent Na^+/H^+ inhibitor with good tolerability and kinetic properties, was chosen for this study.

This study investigated whether HOE642 was able to improve donor heart quality in a setting that closely mimicked the clinical situation of heart transplantation. This differs in two important aspects from previously published studies. First, by the use of hypothermic cardioplegia, the heart is largely protected against the deleterious effects of ischaemia for several hours. Therefore, a beneficial effect of the studied drug is less evident than in studies in which severe ischaemic damage is induced by, for example normothermic ischaemia, but is of more clinical relevance for heart preservation. Furthermore, it is uncertain whether the drug maintains its cardioprotective effect at low temperature. The only study where a Na^+/H^+ inhibitor was tested in a situation of cold cardioplegia is that of Myers and Karmazyn [28], but this was in a crystalloid perfused isolated rabbit heart. Secondly, brain death can induce myocardial damage, with a potentially different reaction to the cardioprotective drug. Brain death might also increase the vulnerability of the hearts to the deleterious effects of ischaemia. Therefore, Bittner *et al.* [29] stated that all preservation and transplantation studies should include a group with brain-dead donor hearts.

In this study the effect of treating both donor and recipient with 2 mg/kg of HOE642 on myocardial function after transplantation was determined. Brain-dead and non-brain-dead dogs were used as heart donors.

Materials and methods

The recovery of myocardial function was studied in a canine model of cardiac transplantation after 4 h preservation. Hearts were retrieved from brain-dead and non-brain-dead donors. All animals received humane care in compliance with the *Principles of Laboratory Animal Care* formulated by the Institute of Laboratory Animal Resources and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Anaesthesia

Adult mongrel dogs of bodyweight 22–37 kg, were anaesthetized by intramuscular injection of piritramide (Dipidolor®) 5 mg/kg followed by intravenous administration of sodium pentobarbital 20–25 mg/kg. After endotracheal intubation, mechanical ventilation was started with a volume-controlled respirator. Arterial blood gases and pH were kept within physiological limits by adjustment of the ventilation and by intravenous administration of sodium bicarbonate.

Surgical procedures

Four groups were studied: untreated brain-dead donors (group 1; $n = 5$); untreated non-brain-dead donors (group 2; $n = 5$); treated brain-dead donors and treated recipients (HOE642 2 mg/kg, group 3; $n = 5$); and treated non-brain-dead donors and treated recipients (group 4; $n = 6$).

Induction of brain death in donors

Fluid-filled catheters were inserted in the right brachial artery and vena cava superior to monitor arterial blood pressure and central venous pressure. A Swan–Ganz catheter was inserted through the right jugular vein to measure the cardiac output and pulmonary capillary wedge pressure at regular intervals. ECG lead II was continuously monitored.

In groups 2 and 4, a Foley catheter was placed in the epidural space through a burr hole in the right parietal bone. Brain death was induced by sudden inflation of the epidural balloon with 15 ml of saline solution, using a model that reliably induces brain death [30]. After brain death, the haemodynamic parameters mean arterial blood pressure, mean central venous pressure, mean pulmonary artery pressure, mean pulmonary capillary wedge pressure, cardiac output and heart rate of the donor were recorded for 1 h. Mean central venous pressure of the donor was maintained above 5 cmH_20 by fluid replacement with Ringer's lactate after brain death.

Harvesting and preservation of donor hearts

One hour after brain death, or in time-matched controls, a sternotomy was performed, the heart was suspended in a pericardial cradle and 300 IU/kg of heparin were administered. In groups 3 and 4, donors were treated with HOE642 (2 mg/kg i.v.) 15 min before the start of ischaemia. The superior vena cava was closed and 1 l of hypothermic (2–4°C) NIH2 cardioplegic solution (*Table 1*) was infused into the aortic root through a 16-G cannula after cross-clamping the ascending aorta. The heart was vented by incising the inferior vena cava and right superior pulmonary vein distally. The heart then was immersed in 4°C Ringer's lactate solution during

Table 1 Composition of hyperkalaemic cardioplegic solution (NIH-2) used

Component	Concentration
Na^{2+}	97 mEq/l
K +	14.89 mEq/l
Cl^-	91.9 mEq/l
Ca^{2+}	0.068 mEq/l
HCO_3^-	20 mEq/l
Glucose monohydrate	27.5 g/l
Mannitol	12.5 g/l
Lidocaine-HCl	0.123 g/l

infusion of cardioplegic solution, and was excised and immediately stored in the same cold (2–4°C) solution.

During the period of immersion, tilting disc prostheses (Björk–Shiley-monostrut #17, 23) were implanted in the aortic position along the subcoronary ring and in the mitral position along the mitral annulus by continuous suture with Prolene 3/0 after removal of native aortic and mitral valve leaflets. Myocardial temperature was continuously monitored (E5000; Eirelec Limited, Dundalk, Ireland) by a thermistor probe (TMPM; Shiley Inc., USA) in the interventricular septum during the valve implantation procedures.

The heart was placed in a plastic bag containing cold cardioplegic solution and packed in a conventional way with plastic bags and crushed ice. The isothermal box with the heart was kept in the cold room with the temperature between 2°C and 4°C and preserved for 4 h with the myocardial temperature as low as ± 1.5°C (*Figure 1*).

Orthotopic heart transplantation

Weight-matched recipient dogs were selected. After anaesthesia, 0.1 mg/kg of pancuronium bromide (Pavulon®) and 1 mg/kg of piritramide were administered additionally. The temperatures of the oesophagus and rectum were monitored.

Mean arterial blood pressure and mean central venous pressure were followed as described before. The heart was exposed through a median sternotomy and pericardiotomy. A balloon-tipped pulmonary artery catheter was inserted via the right ventricular outflow tract to measure the pretransplant cardiac output.

After heparinization (300 IU/kg) and administration of methyl prednisolone (5 mg/kg), both femoral arteries were cannulated with a 12-Fr aortic cannula (USCI® Pediatric Perfusion Cannula; William Harvey®, Santa Ana, California, USA) to provide arterial inflow. The superior and inferior caval veins were cannulated separately with 21 Fr venous catheters (Polystan A/S, Walgerholm, Vaerlse, Denmark) for venous drainage to the cardiopulmonary bypass system. The azygous vein was ligated.

Cardiopulmonary bypass was carried out using a centrifugal pump (Bio-Medicus®; Bio-Medicus, Eden Prairie, Minnesota, USA), two roller pumps (Sarns®, Ann Arbor, Michigan, USA), a membrane oxygenator (Univox® Membrane Oxygenation Sys-

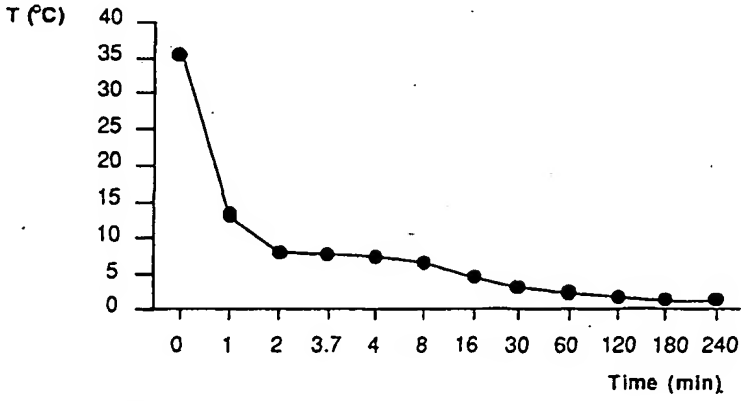

Figure 1 Myocardial temperatures during ischaemia and preservation for 4 hours

tem, Baxter Healthcare Corporation, Bentley Laboratories Division, Irvine, California, USA) with a venous reservoir (Minimax 1316 Filtered Hardshell Reservoir; Medtronic Cardiopulmonary, Anaheim, California, USA), and a heat exchanger (Blanketrol; Cincinnati Subzero Products, Inc., Cincinnati, Ohio, USA). The cardiopulmonary bypass system was primed with 500 ml of plasma-lyte, 500 ml of Geloplasma, and 400 ml of whole blood obtained from donor dogs. Blood flow was maintained at 2.4 l/min per m^2 and gas flow (air and oxygen) between 3 and 4 l/min during cardiopulmonary bypass.

After systemic cooling to 25°C of rectal temperature, the ascending aorta was cross-clamped. The heart was explanted, leaving both atria partially in place. The donor heart was transplanted orthotopically while ice slush was used topically.

In groups 3 and 4, 2 mg/kg of HOE642 was given intravenously to the recipient dog 15 min before reperfusion of the transplanted heart.

With the release of the cross-clamp on the ascending aorta, the coronary flow was re-established and de-air procedures were performed through the ascending aorta and left ventricle. The transplanted heart was reperfused for 60 min while venting the left ventricle for the drainage of Thebesian venous flow. Electrical defibrillation was applied if necessary when the myocardial temperature was above 31°C. Afterwards the ventricle was paced at a rate of 110 beats/min.

Functional measurements

At the end of reperfusion, a latex balloon containing a 7 Fr Millar Mikro-Tip® catheter pressure transducer (Millar Instruments, Houston, Texas, USA) was introduced into the left ventricle through the apex and used to measure isovolumetric pressures at different filling volumes. The left ventricle was vented and right atrial blood was drained by gravity for coronary flow drainage during total cardiopulmonary bypass. The ventricular pressure was measured with 5-ml increments of balloon volume from 5 ml to 35 ml. As a result of implanted valves being used, balloon herniation was absent during the whole functional measurements. A balloon slightly bigger than the left ventricular cavity was chosen to measure ventricular wall tension accurately [31].

Measurement of cardiac work performance after weaning from cardiopulmonary bypass

Weaning from cardiopulmonary bypass was accomplished without positive inotropic support. Cardiac output was measured by thermodilution, calculated by a Cardiac Output Computer, COM-1® (American Edwards Laboratories, Irvine, California, USA), and the mean of triplicate measurements was used.

Measurement of heart weights

At the end of the experiment, transplanted hearts were excised and the whole left ventricular wall, including the septum, was weighed to obtain the wet weight. The specimen was frozen at -30°C and dried in a vacuum pump (ALPHA 1-5; Ehniss Medizinischer Apparatebau, 3360 Osterode am Harz, Germany) for 48 h to obtain the dry weight.

Ultrastructural examination

Specimens of left ventricular transmural needle biopsies that were taken at the end of reperfusion of the transplanted heart were fixed using a 2% glutaraldehyde solution in Soerensen phosphate buffer (pH7.4). After the tissues were sliced, they were postfixed and immersed in a 2% pyroantimonate solution for Ca^{2+} staining. After dehydration, the samples were embedded in epoxy and stained with uranyl acetate and lead citrate. All samples were examined using transmission electron microscopy.

Data analysis

Myocardial water content

The percentage of myocardial water content (MWC) is calculated using the following formula:

$$MWC = (1 - \textit{dry/wet weight ratio}) \times 100\%$$

Left Ventricular compliance as end-diastolic pressure–volume relationship (Figure 2)

For the comparison of data between hearts of different size, left ventricular volume data were nor-

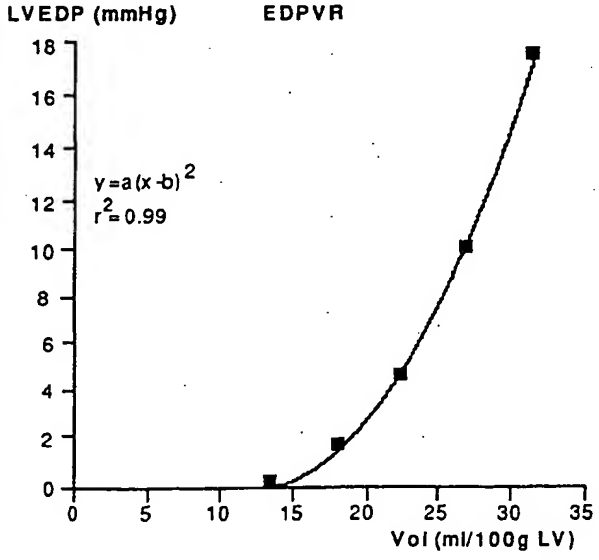

Figure 2 One of the polynomial curve fittings to end-diastolic pressure-volume relationship: $a = 0.004993117$, $b = -12.71569$

malized to a heart weight of 100 g [32, 33] with the following formula:

$$Volume\ normalized = Volume\ measured \times (100/HW)$$

where HW is wet heart weight.

Pressure–volume data were analysed by dividing the obtained pressures into seven 5-ml increments of normalized volume. Each diastolic pressure and volume were plotted and the second-order polynomial curves with two parameters were fitted to the end-systolic pressure–volume relationship data by commercial software (Delta Graph Pro 3.0; Delta-Point, Inc., Monterey, California, USA)with a personal computer, Apple Macintosh ™ PowerBook PowerPC 5300cs using the following equation [34, 35]:

$$P_{ed} = a(V_{ed} - b)^2$$

$$a = dP/dV/2(Ved - b)$$

where P_{ed} is end-diastolic pressure, a is normalized slope, V_{ed} is normalized end-diastolic volume, and b is the volume at zero pressure. This equation includes a zero value for P_{ed} (for $V_{ed} = b$); but excludes negative values and pressure values on normalized volume < 10 ml for the accuracy of left ventrical volume [31, 36].

Left ventrical performance as pressure–volume relationship (Figure 3)

Peak developed pressure and volumes were obtained by calculating and plotting the difference between end-systolic pressure–volume relationship and end-diastolic pressure–volume relationship. A linear function with two parameters was fitted to this plot using the following equation;

$$PDP = aV + b$$

where PDP is peak developed pressure, a is the slope of the regression line, V is normalized ventricular volume, and b the developed pressure in the left ventricle at zero volume.

Cardiac work performance (cardiac index) after weaning from cardiopulmonary bypass

Cardiac index (CI) after weaning from cardiopulmonary bypass is calculated as following formula:

$$CI = Cardiac\ output/0.4 + 0.02 \times body\ weight\ (l/min/m^2)$$

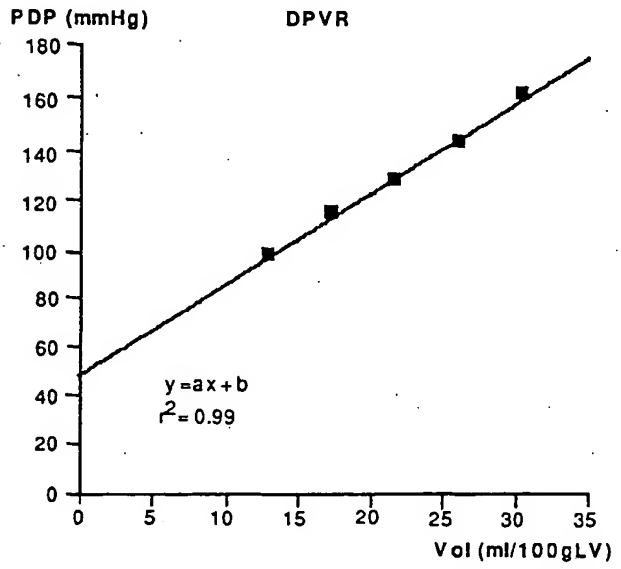

Figure 3 One of the linear curve fittings to end-diastolic pressure-volume relationship; a = 3.763392, b = 48.06.

Statistical analysis

Data are expressed as absolute numbers or mean (s.e.m.). Multiple group comparisons were performed by analysis of variance (ANOVA) with Bonferroni correction for Posthoc testing. The means of each group were compared by unpaired two-tailed Student's t-test. Statistical analysis was performed with Statistica v. 4.5 statistical software (Statsoft, Tulso, Calif., USA) and differences were considered significant at the probability level < 0.05.

Results

Myocardial temperatures

Myocardial temperature fell abruptly to ± 10°C during the first 1–2 min and then gradually decreased. Mean infusion time of the cardioplegic solution was 3.7 min with a mean myocardial temperature of 7.5°C at the end of infusion (*Figure 1*). Implantation of the tilting disc prostheses in the mitral and aortic position was performed between 16 and 60 min after ischaemia with the myocardial temperature below 4°C as shown in *Figure 1*. After 3 h of preservation, the myocardial temperature was always approximately 1.5°C.

Left ventricle compliance (*Figure 4*)

HOE642 significantly reduced the stiffness coefficient a (as defined in the equation above). In hearts from brain-dead donors, HOE642 treatment reduced a from 0.127(0.006) to 0.072(0.016) (P = 0.005). Non-brain-dead donor hearts were also more compliant when treated with HOE642 (a = 0.152(0.013) versus 0.096(0.019); P = 0.015). No interaction was detected between the drug and

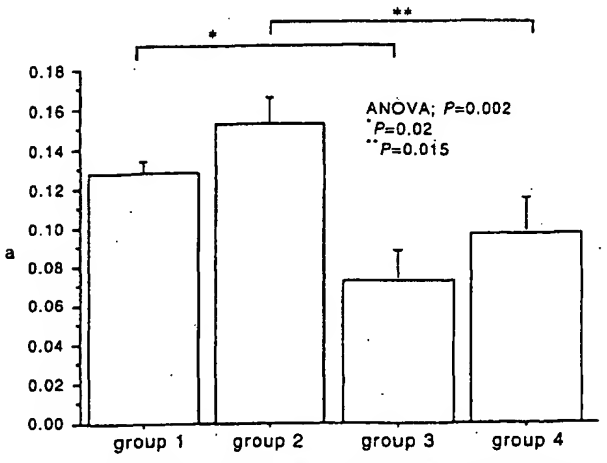

Figure 4 Left ventricle stiffness coefficients (a): = stiffness coefficients were significantly different between groups 1 and 3 (0.127(0.006) versus 0.072(0.016); $P = 0.02$)), and between groups 2 and 4 (0.152(0.013) versus 0.096(0.019); $P = 0.015$)). Data are means (bar = s.e.m.)

whether or not the donor was brain-dead, implying equal effectiveness of the drug in both situations.

Left ventricle performance (*Figure 5*)

Although the groups treated with HOE642 performed slightly better, no significant differences could be detected between groups (ANOVA; $P = 0.098$).

Myocardial water content (*Figure 6*)

The myocardial water content appeared to be the lowest in group 2 (78.62(0.25)%). However, no significant differences were found between groups (ANOVA;$P = 0.38$).

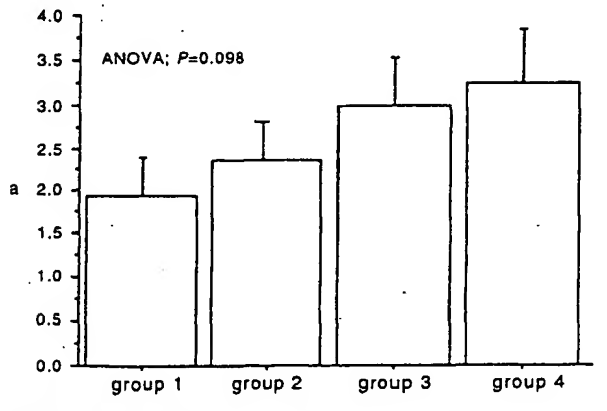

Figure 5 Left ventricle performance (calculated as the slope of the left ventricle developed pressure-volume relationship. There were no significant differences among the preserved donor groups (ANOVA;$P = 0.098$). Data are means (bar = s.e.m.)

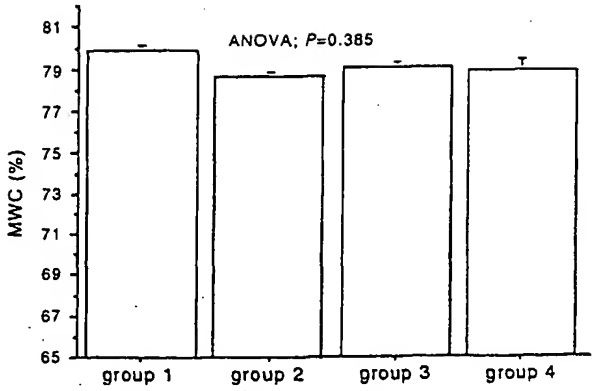

Figure 6 Myocardial water content (%); there were no significant differences between the groups (ANOVA; $P = 0.385$). Data are means (bar = s.e.m.)

Cardiac index after weaning (*Figure 7* and *Figure 8*)

All hearts could be weaned from cardiopulmonary bypass without inotropic support. Although hearts in group 4 (non-brain-dead donors and recipients treated by inhibitor) appeared to have the highest cardiac index, no significant differences were observed between groups (ANOVA; $P = 0.79$).

Ultrastructural examination

No irreversible damage in ultrastructure, such as mitochondrial rupture, swelling or calcium deposits was detected. There was no visible inter- or intracellular oedema, nor depleted glycogen in donor myocardium after transplantation in any group (*Figure 9*).

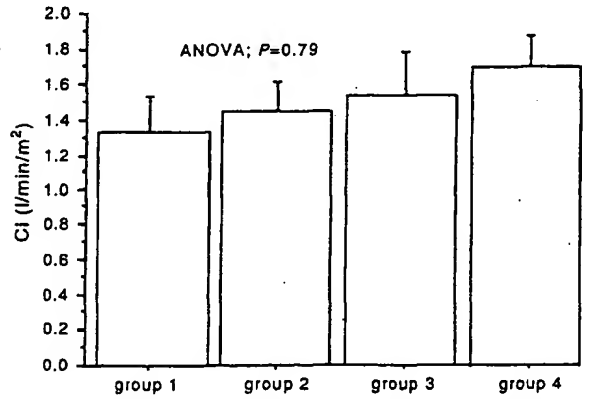

Figure 7 Mean cardiac index after weaning of transplanted hearts from cardiopulmonary bypass; no significant differences were demonstrated (ANOVA; $P = 0.79$). Data are means (bar = s.e.m.)

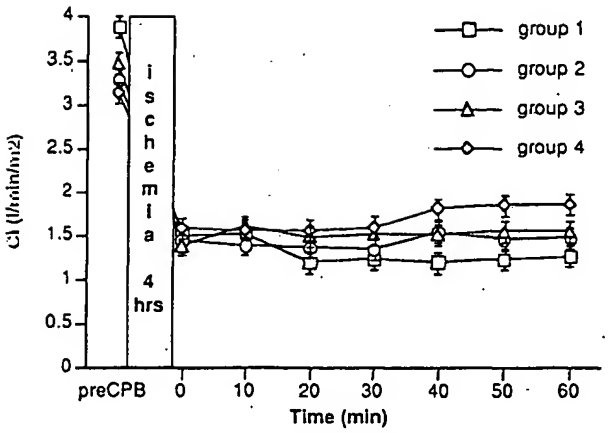

Figure 8 Cardiac index during 60 min after weaning from cardiopulmonary bypass. All groups showed a depressed cardiac index when compared with the pre-cardiopulmonary bypass value. No significant differences were observed between the groups (ANOVA; $P = 0.796$). Values are means (bar = s.e.m.)

Discussion

This study proved the beneficial effect of HOE642 on myocardial compliance in a setting which closely mimicked clinical heart transplantation. An optimal donor management [37] together with an improved technique of myocardial protection during preservation and reperfusion, might permit the pool of transplantable hearts to be increased and the incidence of early graft failure to be reduced [1].

A number of studies have demonstrated that pharmacological inhibition of Na^+/H^+ exchanger exerts a cardioprotective effect on the reperfused myocardium as shown by improved functional recovery [20, 22, 25, 38] reduced contracture [11, 38], diminished arrhythmias [9, 10, 12, 38], and preserved ultrastructures [38]. There have been extensive studies for the cardioprotective effect of Na^+/H^+ inhibitors, such as amiloride derivatives which showed limited application. The present authors selected a new potent and highly selective Na^+/H^+ inhibitor, HOE642, with less toxicity compared with HOE694.

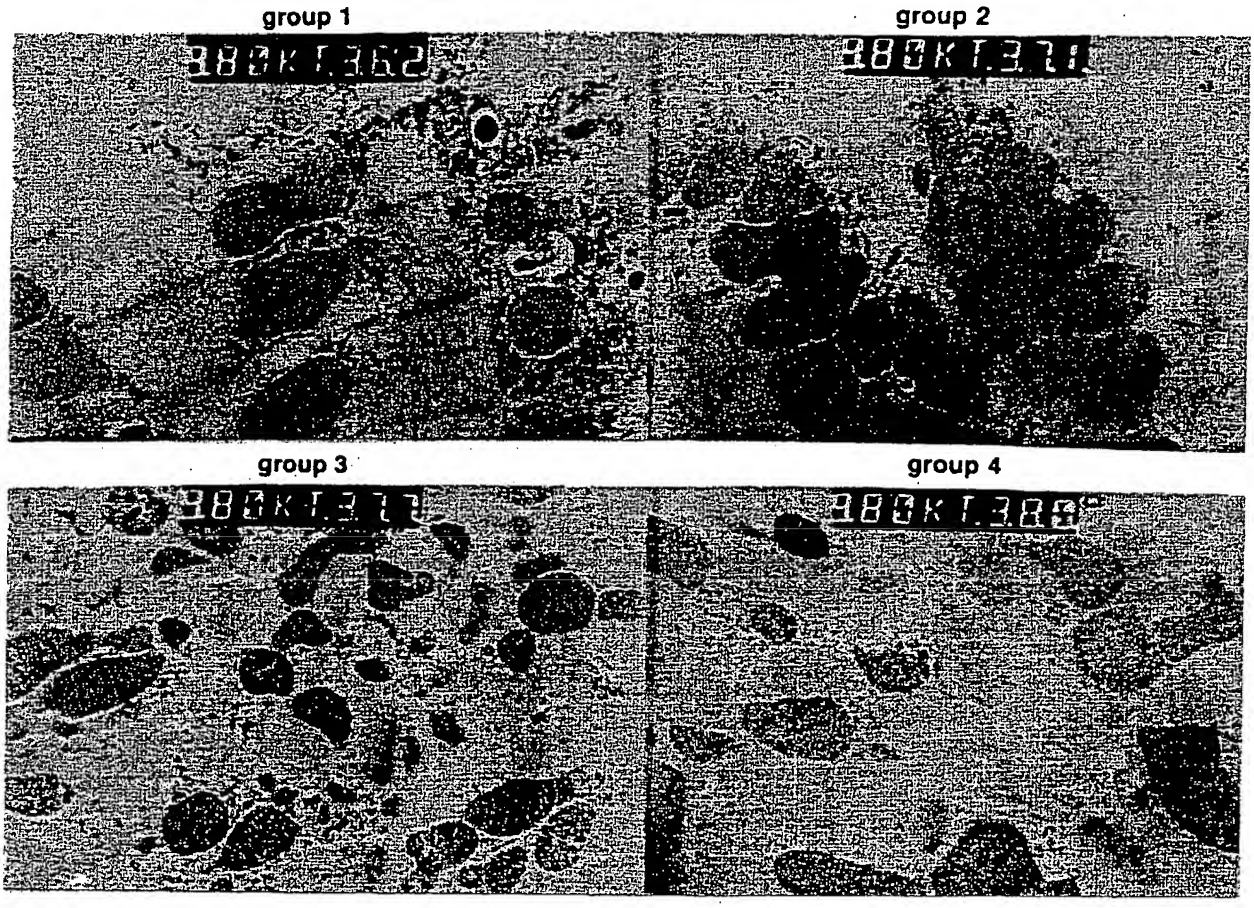

Figure 9 Ultrastructure of myocardium without irreversible injuries. Examples of groups 1, 2, 3, and 4 are shown in panels a, b, c and d respectively

Also in the situation of the potentially altered cellular response under profound hypothermic conditions as opposed to normothermic ischaemia-as has been used in virtually all other studies involving Na^+/H^+ inhibition [28]-these agents seem to be cardioprotective. However, the study concerning the cardioprotective effect of this inhibition in the brain-dead donor heart which is preserved in profound hypothermia and orthotopically transplanted has not yet been performed to the authors' knowledge. They used the new Na^+/H^+ inhibitor, HOE642 in the brain-dead donor and recipient to assess the cardioprotective effects in transplanted canine myocardium after 4 h global ischaemia to simulate clinical practice as closely as possible.

When both donor and recipient are treated with HOE642 both in the brain-death and non-brain-dead group, significantly lower myocardial stiffness is shown after transplantation than in the untreated group. However, there were no significant differences for the left ventricle systolic performance and myocardial water content among the groups.

Myocardial stiffness is known to be influenced by myocardial contracture and/or oedema [8, 13–17]. In the present study, significantly different stiffness coefficients without difference in myocardial water content among the groups suggest that the improvement in myocardial compliance in the treated groups is mainly affected by myocardial contracture.

All groups showed successful weaning from cardiopulmonary bypass without inotropic support such as dopamine and/or dobutamine, and well-preserved ultrastructure without irreversible damages. However, the postweaning myocardial cardiac index seemed slightly better in treated groups than in untreated groups.

Brain death was shown to induce myocardial damage and impairment of ventricular function [29, 30]. In this study, however, the brain death group did not show significantly worse recovery of myocardial function or more ultrastructural injury than did the non-brain-dead group. Although focal necrosis in myocardium resulting from brain death is observed clinically in some cases, most transplantations using brain-dead donor hearts fortunately have been very successful. If the overall results of heart transplantation need to be improved, the best way to improve donor heart quality may be to optimize preservation and reperfusion techniques. In that respect, HOE642 is probably a useful drug.

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